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The FMRFamide-Related Peptides F1 and F2 Alter Hemolymph Distribution and Cardiac Output in the Crab *Cancer magister*

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Abstract. The FMRFamide-related peptides F1 and F2, originally isolated from lobster pericardial organs, have been shown to exert cardioexcitatory effects on isolated or semi-isolated crustacean hearts. The present study sought to determine the *in vivo* effects of F1 and F2 on cardiac and circulatory performance of *Cancer magister* using a pulsed-Doppler technique. In general the effects of F1 and F2 were similar; however, F1 was more potent and its effects were of longer duration than those exerted by F2. Infusion of either F1 or F2 caused a decrease in heart rate and subsequent periods of acardia. These decreases in rate occurred concurrently with a short-term increase in stroke volume of the heart, followed by a longer-term decrease in stroke volume. Hemolymph flow rates through the anterior aorta, anterolateral arteries, sternal artery, and posterior aorta also showed the same trend, with an initial short-term increase in flow rate followed by a longer-term decrease with periods of ischemia. Hemolymph flow through the paired hepatic arteries simply decreased, but recovery to pretreatment levels was faster than in the other arterial systems. Threshold for these responses occurred at circulating concentrations between 10^{-9} mol · l^{-1} and 10^{-8} mol · l^{-1} for F1 and somewhat higher, between 10^{-8} mol · l^{-1} and 10^{-7} mol · l^{-1}, for F2.

Introduction

The neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) was first isolated and sequenced from the clam *Macrocallista nimbosa* (Price and Greenberg, 1977). FMRFamide is now known to belong a large family of neuropeptides, collectively called the FMRFamide-related peptides (FaRPs), that share the sequence Arg-Phe-NH_2 and exist throughout the invertebrate and vertebrate kingdom (for reviews see Greenberg and Price, 1983; Raffa, 1988; Price and Greenberg, 1989). FaRPs generally function as neurohormones or neurotransmitters; their action on the cardiovascular system is predominantly excitatory, but inhibitory or biphasic effects occur in some species (*e.g.*, Painter and Greenberg, 1982; Cuthbert and Evans, 1989; Price *et al.*, 1990; Duve *et al.*, 1993; Lesser and Greenberg, 1993).

In crustaceans, FMRFamide-like immunoreactivity has been found throughout the nervous system, the highest amounts being concentrated in the pericardial organs (Kobierski *et al.*, 1987; Marder *et al.*, 1987; Trimmer *et al.*, 1987; Krajniak, 1991; Mercier *et al.*, 1991). Two of these peptides have been isolated and sequenced from the pericardial organs of the lobster *Homarus americanus* (Trimmer *et al.*, 1987) and have been identified as having the sequences TNRNFLFRamide (F1) and SDRNFLFRamide (F2). Both have since been reported in the stomatogastric system of the rock crab *Cancer borealis* (Weimann *et al.*, 1993). The high concentration of these peptides in the pericardial organs suggests that they may be released into the circulatory system to directly affect the heart or cardioarterial valves. Both F1 and F2 excite isolated lobster (*H. americanus;* Kravitz *et al.*, 1987) and blue crab (*Callinectes sapidus;* Krajniak, 1991) hearts, and increase amplitude and frequency of hearts isolated from crayfish (*Procambarus clarkii;* Mercier and Russenes, 1992) and shore crab (*Carcinus maenas;* Wilkens and McMahon, 1992). In the lobster and crayfish, these pep-

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Abbreviations. FaRPs, FMRFamide-related peptides; F1, TNRNFLRFamide; F2, SDRNFLFRamide; CNS, central nervous system.

tides restrict hemolymph flow by their actions on the cardioarterial valves (J. L. Wilkens, pers. comm.).

In addition to their action on the heart, F1 and F2 also activate the pyloric and gastric rhythms in *Cancer borealis* (Weimann *et al.*, 1993), and F1 has powerful effects on the phasic extensor muscle of the lobster (Pasztor and Golas, 1993). There are very few reports of the effects of neurohormones on the cardiovascular dynamics of whole-animal preparations. We recently developed a pulsed-Doppler technique for measuring blood flow and cardiac output in *Cancer magister in vivo* (Airriess *et al.*, 1994). This technique is minimally invasive and allows simultaneous measurement of blood flow in all arteries leaving the heart. This permits calculation of cardiac output which, when divided by heart rate, yields stroke volume.

A number of amine and peptide neurohormones have been shown to have distinctive effects on cardiac and circulatory function in crustaceans *in vivo* compared with isolated or semi-isolated preparations (McMahon and Reiber, 1991; Airriess and McMahon, 1992; McGaw *et al.*, 1994a). Thus the aim of the present study was to determine the effects of the FMRFamide-related peptides F1 and F2 on hemolymph flow and cardiac output *in vivo* in the Dungeness crab *C. magister*.

Materials and Methods

Adult male intermolt *Cancer magister* weighing 600–850 g were purchased from local fishermen and held at Bamfield Marine Station, British Columbia, Canada, in filtered running seawater at a temperature of 12 ± 1°C and a salinity of 33 ± 1% for at least 1 week prior to experimentation. Crabs were usually fed chopped fish twice a week but were isolated from food supplies for 2 days prior to experimentation.

A 545C-4 directional pulsed-Doppler flowmeter (Bioengineering, University of Iowa) was used to measure hemolymph velocity in each of the major arteries. This technique of minimally invasive flow measurement and probe calibration is described in detail in Airriess and McMahon (1994) and Airriess *et al.* (1994). Briefly, piezoelectric crystal probes (Iowa Doppler Products, Crystal Biotech) were implanted in grooves abraded to the dermis of the carapace directly above each artery except the sternal and hepatic arteries, which were monitored by means of internal catheter mounted probes. Hemolymph loss during the latter procedure was minimal. Probe performance was first optimized manually, then fine focused electronically to obtain maximum signal amplitude and fixed in place using cyanoacrylate glue and dental wax. Output from the flowmeter was recorded on a Gould 6-channel oscillograph.

Heart rate was determined by counting the peaks on the arterial flow traces. This method, evaluated by Airriess and McMahon (1994), gives values analogous to those produced by the more familiar impedance conversion technique as long as there is measurable hemolymph flow through at least one arterial system. Cardiac output was calculated by summation of the mean flow through each artery (values for paired arteries were doubled), and this value was divided by mean heart rate to obtain a mean value for cardiac stroke volume. Scaphognathite beat frequency was recorded with a hydrostatic pressure transducer (Statham/Gould) connected *via* a saline-filled catheter to the right branchial chamber.

During experiments, crabs were held in a covered acrylic plastic box, dimensions 28 × 20 × 10 cm, that was supplied with a constant flow of aerated seawater. The size of the box allowed the crabs minimum movement and thus restricted damage to the probe implants. After electrodes were implanted, animals were allowed to settle for at least 24 h before experimentation. Experiments were carried out in constant darkness at a temperature of 12 ± 1°C.

F1 was obtained from Bachem Bioscience, Inc., and F2 was a gift from Dr. Joffre Mercier. The peptides were dissolved in *Cancer* saline (Morris and McMahon, 1989) and diluted to achieve final calculated circulating concentrations of 10^{-6} to 10^{-12} mol · l^{-1}. Test solutions were infused directly into the lateral pericardial sinus by means of a chronically implanted polyethylene catheter (PE20). A syringe pump (Sage Instruments) was used to infuse 350 μl of the test solution followed by 150 μl of saline for catheter washout over a 3-min period. This interval was long enough to ensure that the hormone did not reach the pericardial sinus as a concentrated bolus of injectate but was distributed slowly and homogeneously (Airriess and McMahon, 1992). Control infusions were carried out with *Cancer* saline. Each experimental animal received the entire concentration range of either F1 or F2.

Heart rate, hemolymph flow rates, and scaphognathite beat frequency were determined for 11 animals tested with F1 and for 10 animals tested with F2. Recordings were carried out at 10-min intervals during a 30-min control period, during and immediately after infusion of either saline or peptide solution, and at regular intervals after infusion up to a total time of 120 min for each hormone concentration.

One-way analysis of variance with repeated measures design (Potvin *et al.*, 1990) was carried out on the data to test for significant differences between pre- and post-treatment levels; any missing values were statistically estimated (Zar, 1984).

Results

The heart rate, scaphognathite rate, and hemolymph flow through each of the arterial systems leaving the heart

were determined for 11 crabs tested with F1 and 10 crabs tested with F2 (Figs. 1–3 and 8). The figures show mean responses (with SE) to a control infusion of *Cancer* saline and a circulating concentration of F1 and F2 (10^{-7} mol · l^{-1}) that had distinct effects.

Saline infusion of 350 μl caused no significant change (ANOVA $P > 0.05$) in any of the measured cardiovascular parameters (Figs. 1–3 and 8, dashed line). In general, the effects of F1 and F2 were similar, but F1 was more potent and its mediated effects tended to be of longer duration than those elicited by F2.

Infusion of either F1 or F2 caused a decrease in heart rate (Fig. 1a and c) ($F = 6.18$ and 4.47, $P < 0.01$) for up to 60 min after peptide treatment, often with periods of acardia. Both the magnitude and duration of these effects were greater with F1. This response was observed between circulating concentrations of 10^{-9} and 10^{-8} mol · l^{-1} for F1, while the threshold for F2 was somewhat higher, between 10^{-8} and 10^{-7} mol · l^{-1}. This long-term decrease in heart rate was initially accompanied by a short-term increase (5 min) in the stroke volume of the heart. Mean stroke volume increased from about 0.15 ml/beat to 0.28 ml/beat with F1 (Fig. 1b) and from about 0.22 ml/beat to 0.31 ml/beat upon administration of 10^{-7} mol · l^{-1} F2 (Fig. 1d). Thereafter, stroke volume decreased in both cases and pretreatment values were not regained until after the end of the 2-h measurement period.

F1 and F2 also elicited similar responses in patterns of hemolymph flow through each of the five major arterial systems. Hemolymph flow through the anterior aorta (Fig. 2a, d), the left anterolateral artery (Fig. 2b, e), the posterior aorta (Fig. 3a, c), and the sternal artery (Fig. 3b, d) showed a short-term increase (<5 min). These changes proved to be statistically significant in all cases (ANOVA, $P < 0.05$) except for the anterior aorta treated with F2 ($F = 1.40$, $P > 0.05$). Thereafter hemolymph flow in each arterial system decreased significantly and often could not be detected for extended periods. In most animals pretreatment flow levels were not regained within the experimental time period (120 min), and often took 3–4 h to recover fully. This long-term decline in hemolymph flow was most apparent in the anterolateral arteries, which also showed evidence

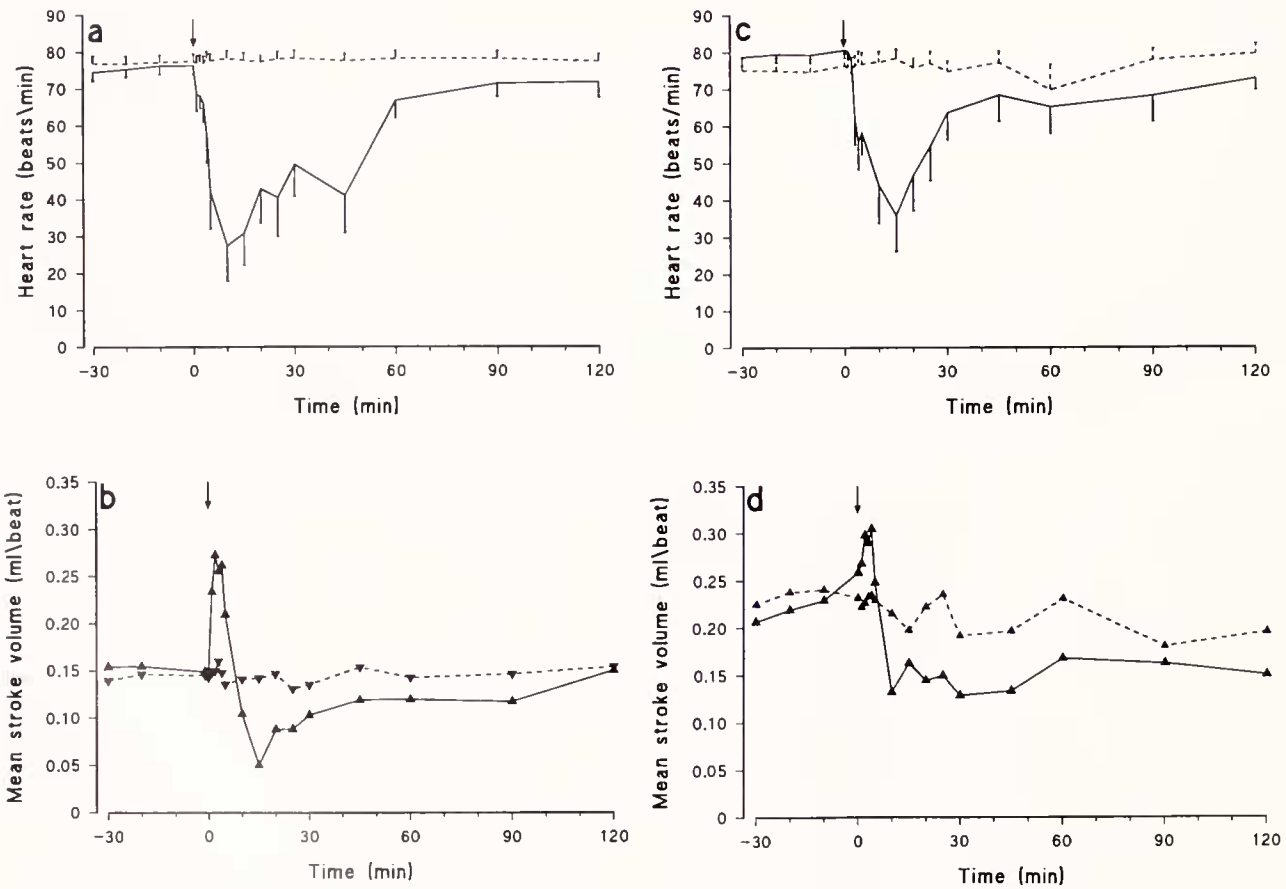


Figure 1. Changes in (a, c) mean heart rate (±SE) and (b, d) mean stroke volume of the heart of *Cancer magister* after infusion of 350 μl of saline (dashed line) and 350 μl of 10^{-7} mol · l^{-1} F1 (a, b) and F2 (c, d) (solid line) at time 0 min (arrow).

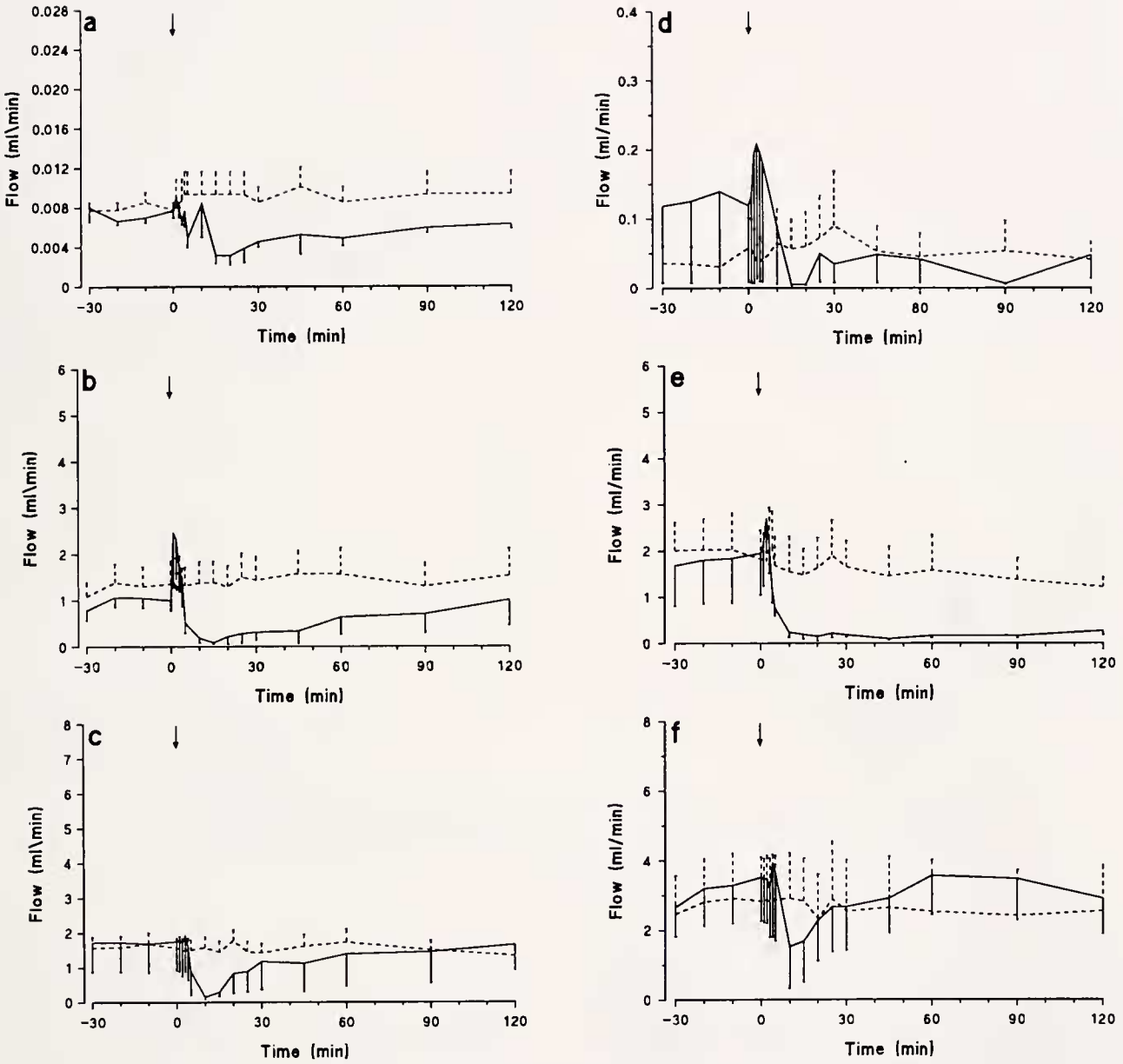


Figure 2. Changes in hemolymph flow (mean ± SE) through the anteriorly directed arteries of *Cancer magister* in response to a 350 μl saline injection (dashed line) or a 350 μl treatment of 10^{-7} mol · l^{-1} F1 or F2 (solid line) at 0 min (arrow). (a, d) Anterior aorta, (b, e) left anterolateral arteries, and (c, f) right hepatic arteries after F1 and F2 infusion respectively.

of a slight reduction in flow at the lower concentrations of F1 and F2 tested (10^{-12} mol · l^{-1} to 10^{-10} mol · l^{-1}).

Hemolymph flow through the right hepatic artery (Fig. 2c, f) did not show the initial short-term increase in response to F1 or F2. Flow through this artery decreased after treatment with F1, although owing to the large variance there was no significant decrease for F2 ($F = 1.49$, $P > 0.05$). The effect of F1 and F2 on hemolymph flow through the hepatic arteries was of shorter duration than in the other arterial systems, and pretreatment levels were usually regained within 30–45 min after hormone infusion.

There was large inter-individual variability in both the duration and the magnitude of hormone-induced changes in hemolymph flow through each arterial system (duration varied from 20 s to 10 min). Because such changes tended to be obscured when shown only as mean responses (Figs. 1–3), representative examples are given in Figures 4 and 5. Increases in flow through each arterial system were not simultaneous. In the majority of animals tested there was

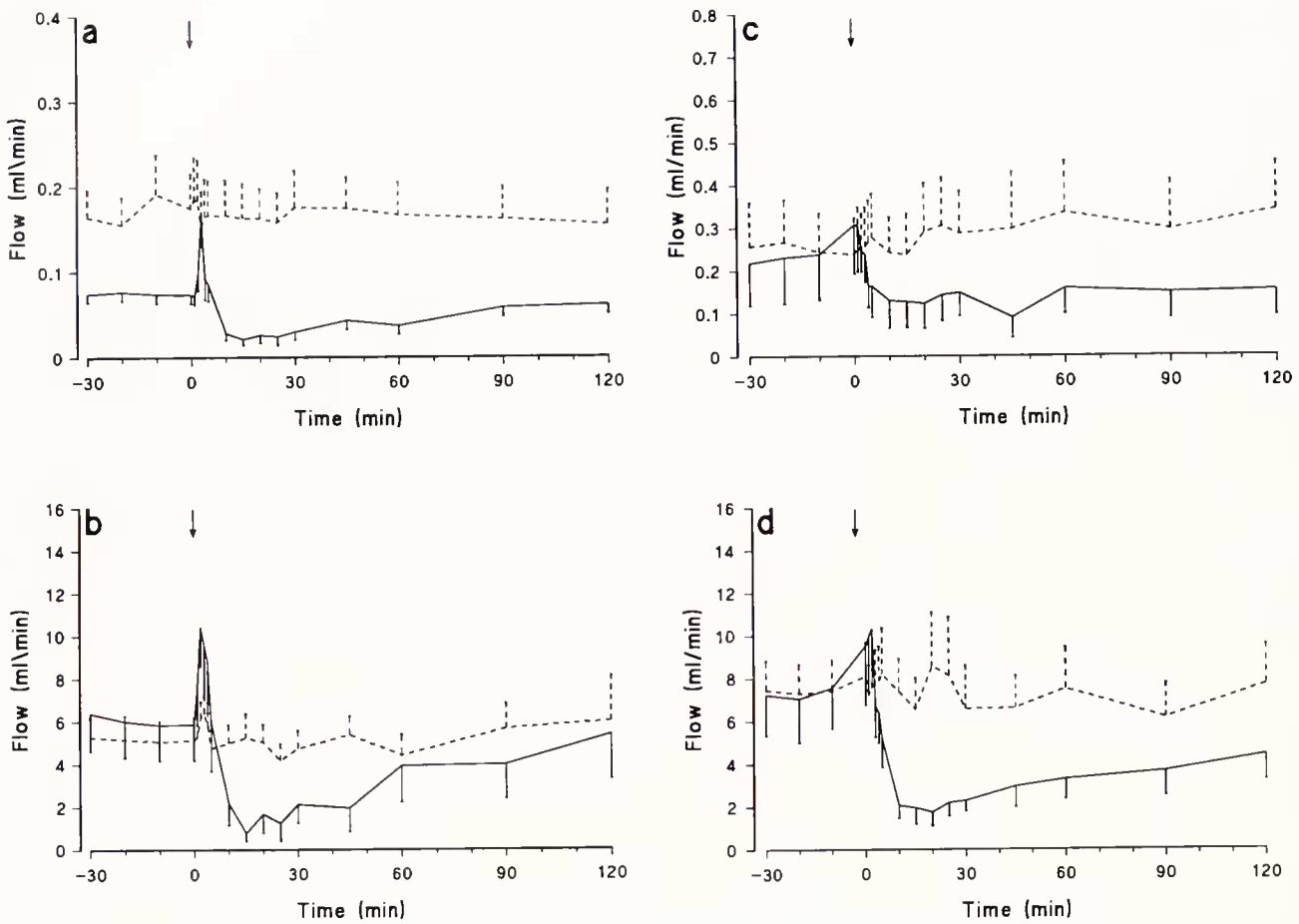


Figure 3. Changes in hemolymph flow (mean ± SE) through the ventrally directed arteries of *Cancer magister* after infusion at 0 min (arrow) of 350 μl of saline (dashed line) or 10^{-7} mol · l^{-1} F1 and F2 respectively (solid line). (a,c) Posterior aorta, and (b, d) sternal artery.

a tendency for the anteriorly directed arteries to be affected first and for the ventrally and posteriorly directed systems to respond slightly later (Fig. 4). After the initial short-term increase in hemolymph flows there was a decrease followed by periods of ischemia (Figs. 4 and 5), and evidence suggested that this interval of both decreased flow and ischemia was dose dependent.

Dose response curves (heart rate, $n = 11$) for F1 and F2 are presented in Figure 6. Heart rate decreased in a simple dose-dependent manner. F1 significantly decreased heart rate at concentrations between 10^{-9} mol · l^{-1} and 10^{-8} mol · l^{-1}, whereas the threshold for F2 was somewhat higher, between 10^{-8} mol · l^{-1} and 10^{-7} mol · l^{-1}.

Total mean cardiac output was calculated by summation of mean flows in all arteries (values for paired arteries were doubled) and expressed as a percentage of flow through each artery (Figs. 7a, b, and c). Saline infusion did not alter the percentage of hemolymph delivered through each system (Fig. 7a). The sternal artery received about 45% of the total cardiac output, about 25% was channeled into each of the hepatic and anterolateral artery systems, and less than 5% was delivered to the smaller-diameter posterior and anterior aortae.

F1 not only produced an overall long-term reduction in cardiac output infusion of 350 μl of 10^{-7} mol · l^{-1}, it also radically altered the distribution of cardiac output (Fig. 7b). The anteriorly directed arteries were affected first: within a minute of hormone infusion a short-term increase in percentage output delivered to the anterolateral arteries and a decrease to the hepatic arteries occurred, while flow through the anterior aorta was routinely low. After about 3 min the percentage of output delivered to the sternal artery and posterior aorta increased for up to 10 min. The longer-term decrease in all arteries except for the hepatic arteries (which recovered to pretreatment flows before the rest of the systems) meant that after 15 min most of the cardiac output was delivered through the hepatic arteries. Similar changes in the distribution of cardiac output resulted from infusion of F2 (Fig. 7c), but the longer duration of flow inhibition in most arteries

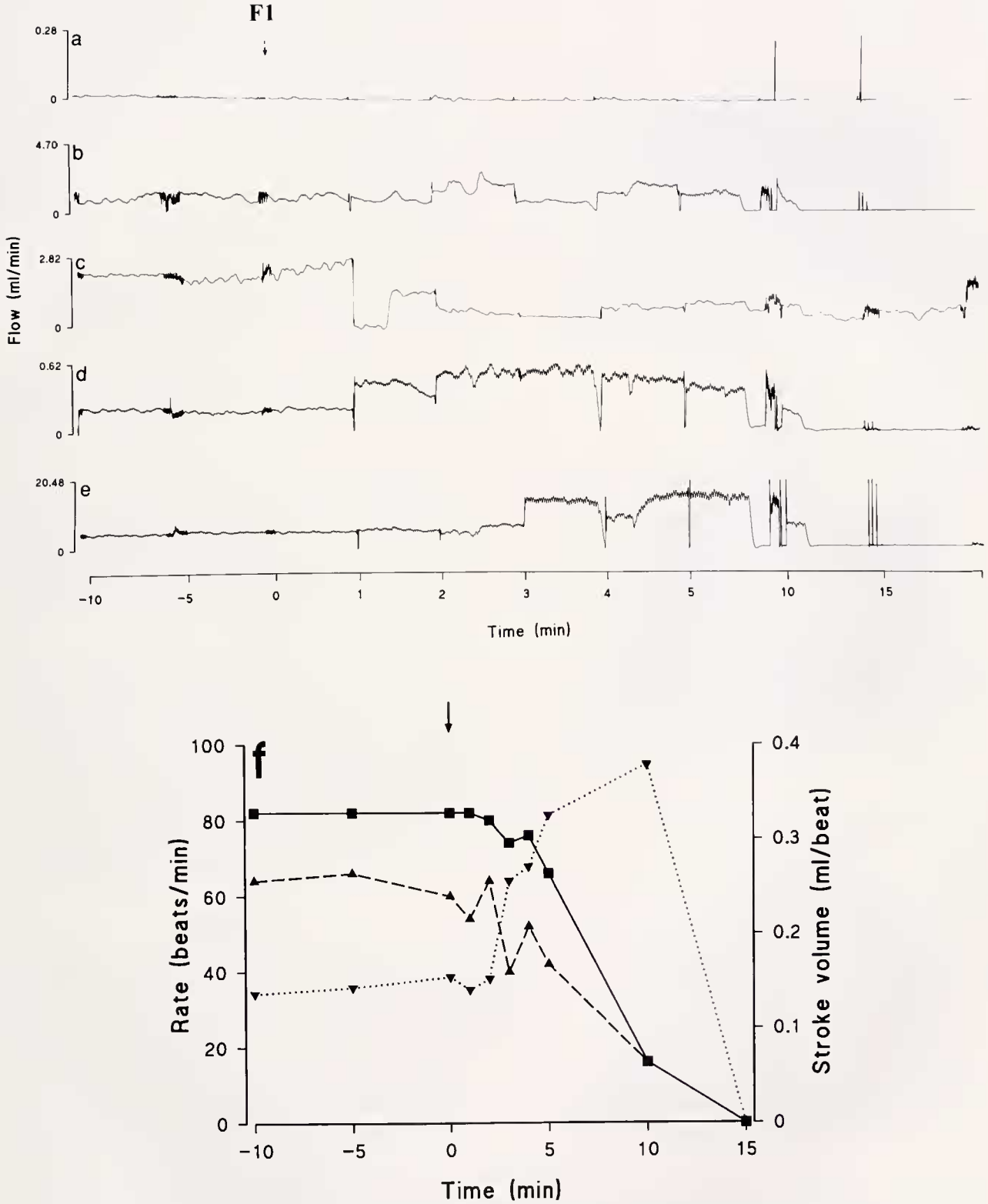
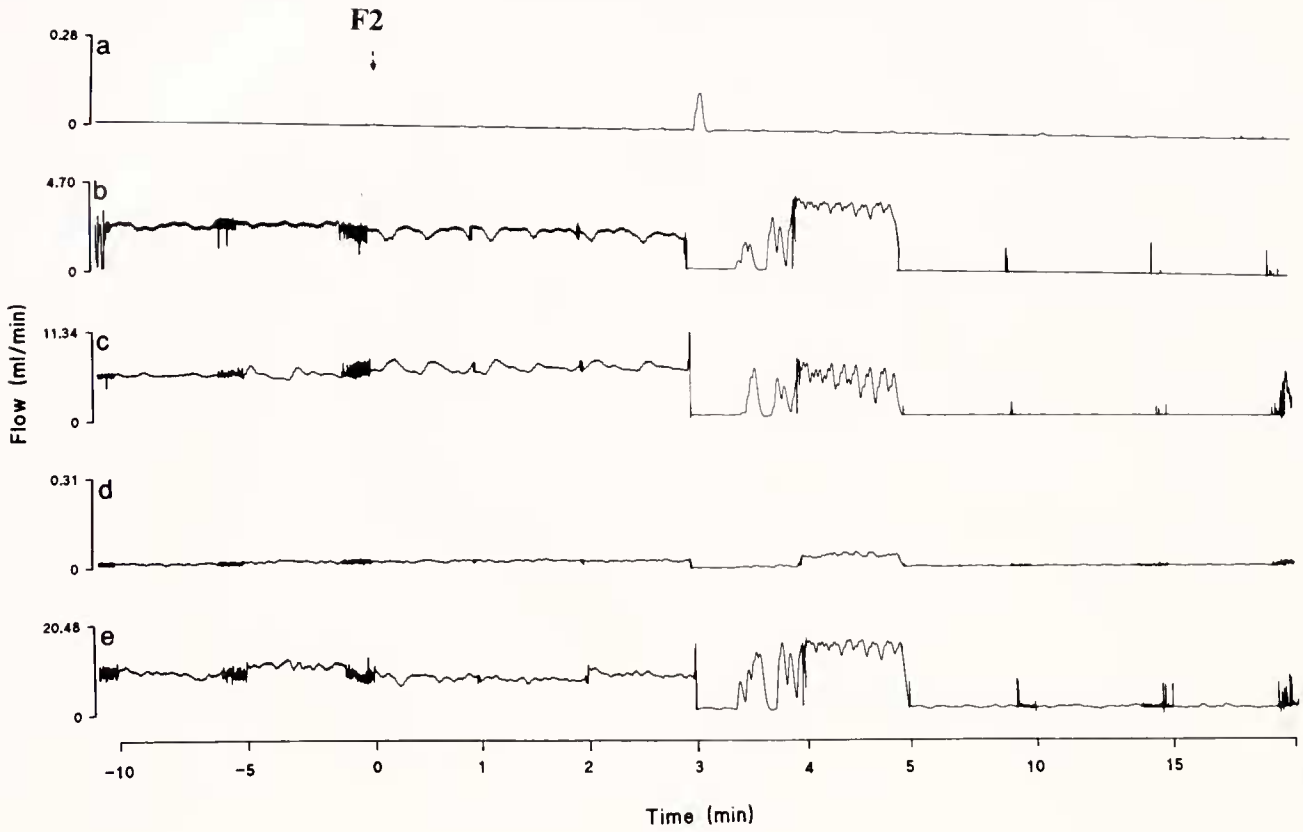


Figure 4. Changes in hemolymph flow (ml/min) and cardiac function in a single specimen of *Cancer magister* after administration of 350 μl of 10^{-7} mol · l^{-1} F1 at 0 min. (a) Anterior aorta, (b) left anterolateral artery, (c) right hepatic artery, (d) posterior aorta, and (e) sternal artery. (f) Represents changes in heart rate (solid), cardiac stroke volume (dotted), and scaphognathite beat frequency (dashed).



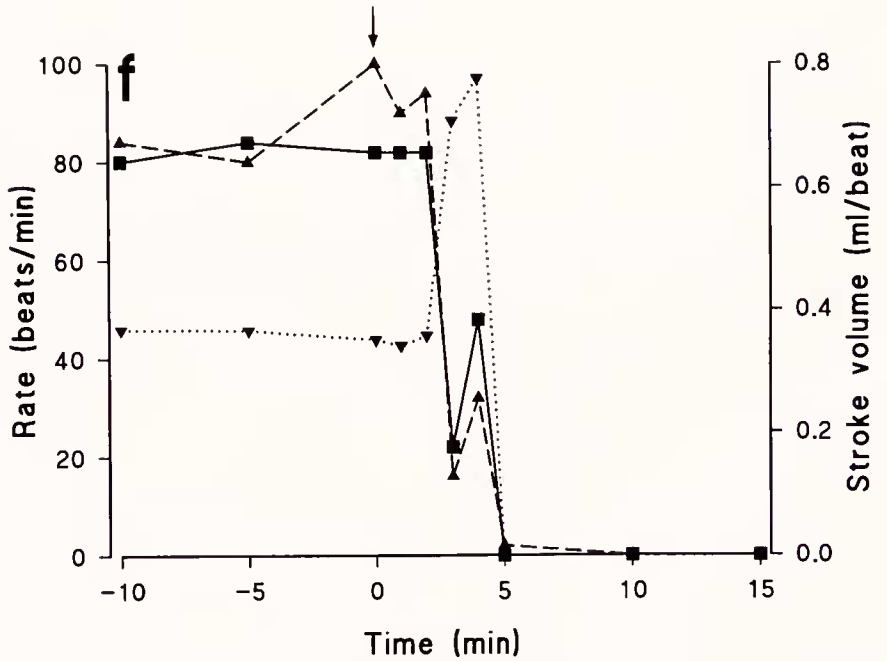


Figure 5. Changes in hemolymph flow (ml/min) and cardiac function in a single specimen of *Cancer magister* after administration of 350 μl of 10^{-7} mol · l^{-1} F2 at 0 min. (a) Anterior aorta, (b) left anterolateral artery, (c) right hepatic artery, (d) posterior aorta, and (e) sternal artery. (f) Represents changes in heart rate (solid), cardiac stroke volume (dotted), and scaphognathite beat frequency (dashed).

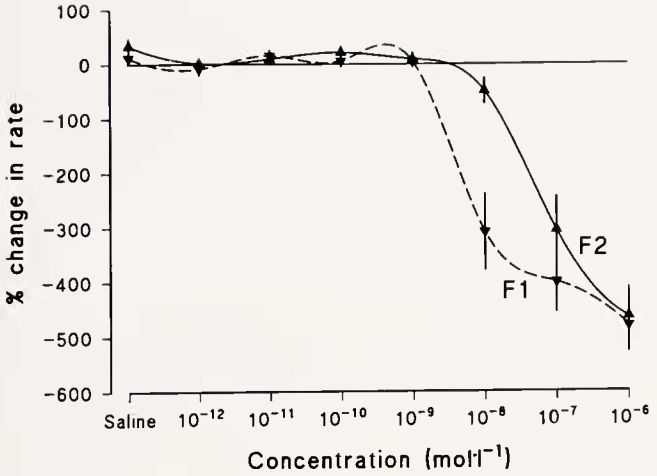


Figure 6. Dose response curves for F1 and F2, depicting cumulative percentage decrease in heart rate (mean ± SE) over a 60-min period after hormone infusion.

extended the period during which most of the flow passed through the hepatic arteries. Note that the hepatic artery flow is not potentiated concurrently. Diversion of a greater percentage of flow into the hepatic arteries at this time is a function of an overall reduction in cardiac output, coupled with a much faster restoration of flow in this arterial system.

The fastest and most dramatic effects of F1 and F2 infusion were on the scaphognathite beat frequency (F = 11.91 and 4.52, $P < 0.01$), and they occurred at lower concentrations (10^{-9} mol · l^{-1} and 10^{-8} mol · l^{-1} respectively) than observed for the cardiac or circulatory indicators. Within 1 min of the start of infusion, beating was disrupted, and subsequently ceased (Fig. 8a, b). As with changes in heart rate, reductions by F1 were of greater magnitude and longer duration than those of F2.

Discussion

Investigation of neuropeptidergic modulation of cardiovascular dynamics in decapod crustaceans has focused largely on the effects on isolated or semi-isolated preparations. The present study provides evidence of the actions of the FaRPs F1 and F2 on heart and circulatory function in whole-animal preparations.

In isolated heart preparations of *Callinectes sapidus* (Krajniak, 1991), *Procambarus clarkii* (Mercier and Russenes, 1992; J. L. Wilkens, pers. comm.), and *Carcinus maenas* (Wilkens and McMahon, 1992), F1 and F2 are positively chronotropic and the thresholds for responses tend to be lower than those reported in the present study. Interestingly, F1 and F2 also excite the isolated heart of *Cancer magister* (McGaw, Wilkens, McMahon, and Airriess, in prep.), elevating frequency for up to 30 min

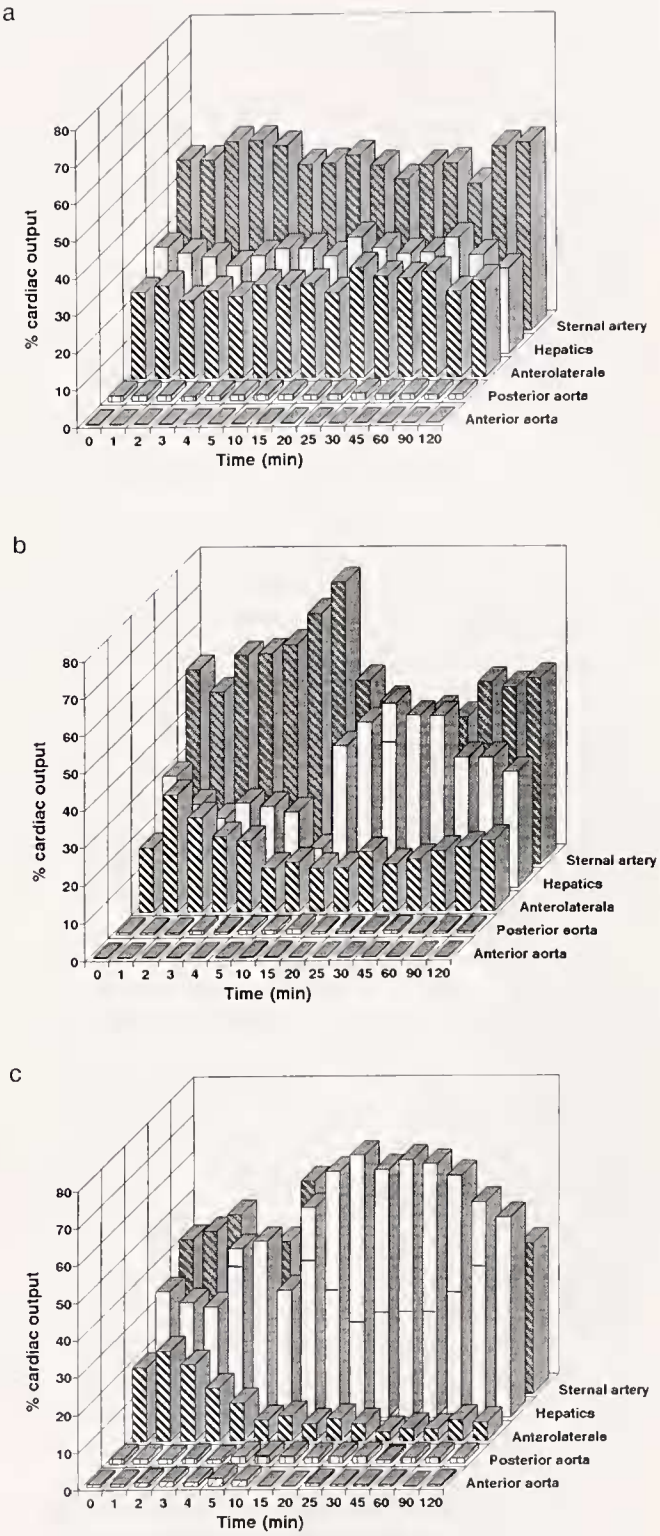


Figure 7. (a) Percentage of total cardiac output delivered through each arterial system after infusion of 350 μl of saline, (b) infusion of 350 μl of 10^{-7} mol · l^{-1} F1, and (c) infusion of 350 μl of 10^{-7} mol · l^{-1} F2. Where values for percentage cardiac output delivered through the sternal artery are obscured, they are shown as horizontal lines on the bars for the hepatic artery.

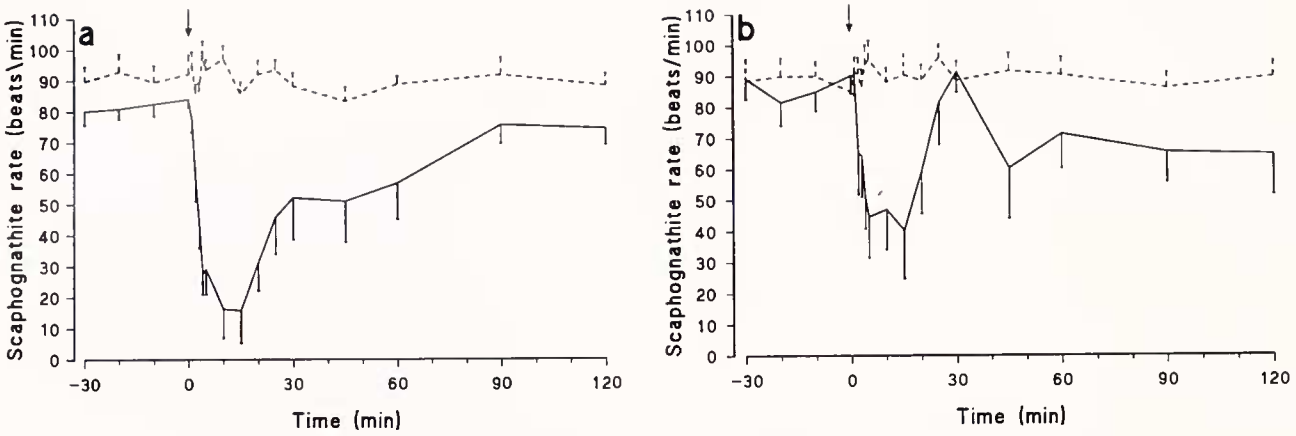


Figure 8. Changes in mean scaphognathite beat frequency after administration of 350 μl of saline (dashed line) or 10^{-7} mol · l^{-1} F1 (a) and F2 (b) (solid line) at time 0 min (arrow).

after hormone application. The threshold for response of the isolated heart is between 10^{-10} mol · l^{-1} and 10^{-9} mol · l^{-1}, which is higher than for intact *C. magister* hearts (Fig. 6). Despite their published reputation as cardioexcitatory peptides, both F1 and F2 induced a rapid bradycardia in intact *C. magister*, followed by periods of acardia (Figs. 1a and c, 4f, and 5f). Similar responses to F1 occur in the lobster *Homarus americanus* (McMahon and Reiber, 1991), although the threshold for inhibition is some 100,000-fold lower, occurring at 10^{-13} mol · l^{-1} instead of at 10^{-8} mol · l^{-1} as in *C. magister*.

F1 and F2 also increase the amplitude of contraction of the isolated heart of *P. clarkii* (Mercier and Russenes, 1992; J. L. Wilkens, pers. comm.). However, this increase in contractility is persistent and, in contrast to the present study, F2 was more potent; similar increases in cardiac stroke volume occur in isolated *C. magister* preparations (McGaw *et al.*, in prep.). In contrast, F1 and F2 have negative inotropic effects on isolated *C. maenas* hearts (Wilkens and McMahon, 1992), whereas no clear pattern is observed in *C. sapidus* (Krajniak, 1991). The action of these hormones on cardiac stroke volume of intact *C. magister* was more complex. Both F1 and F2 caused an initial increase in stroke volume that lasted between 30 s and 10 min. This increase was followed by a longer-term depression; in many cases pretreatment levels of mean stroke volume were not regained within the 120-min test period (Fig. 1b and d).

The discrepancy in cardiac function between isolated and whole-animal preparations suggests that in intact *C. magister* F1 and F2 either exert their effects on the heart by means of inhibitory innervation from the central nervous system (CNS) or cause secondary release of other cardioregulatory hormones (Groome and Watson, 1989). The rapid action of these peptides in disruption and subsequent cessation of scaphognathite beating (Fig. 8) also suggests inhibitory nervous input directly from the CNS.

Modulation of hemolymph flow by F1 and F2 in each of the arterial systems was also complex, and changes were not as uniform as those reported for the heart (Figs. 2 and 3). Duration and magnitude of responses in arterial flow varied quite considerably, not only between animals (Figs. 4 and 5) but even within single animals. Such variability in circulatory function may be associated with the many (but invisible) changes in physiological state that may occur in quiescent animals held under similar conditions (McGaw *et al.*, 1994b). Hemolymph flow increased briefly through all vessels except the paired hepatic arteries and decreased thereafter (Figs. 2 and 3). The initial increase in flow in all arteries except the hepatics must have been brought about by increases in the stroke volume of the heart, since heart rate fell (Fig. 1), and might also involve differential contraction of the cardioarterial valves at the base of each arterial tree. The subsequent decreases in flow resulted from decreases in both heart rate and stroke volume (Fig. 1) and might be associated with simultaneous contraction of all the cardioarterial valves. F2 has not been tested on flow in other crustaceans, but F1 causes similar biphasic effects on hemolymph flow through arteries of the lobster. A short-term increase followed by longer-term decreases and pauses in hemolymph flow occurs in the anterior aorta and the lateral and sternal arteries. A simple decrease occurs in the posterior aorta at higher concentrations (10^{-11} mol · l^{-1} and above). At lower concentrations (10^{-13} mol · l^{-1}) F1 has purely inhibitory effects on lobster hemodynamics (McMahon and Reiber, 1991).

In the present study, the effects of F1 and F2 on the anterolateral arteries were not wholly consistent. In about 75% of the animals tested, flow initially increased; in the

remainder, it just decreased. Similar responses are also reported for the lobster (McMahon and Reiber, 1991). The anterolateral arteries are often subject to periodic cycles of hemolymph flow without any apparent *zeitgeber* in *C. magister* (McGaw *et al.*, 1994b) and, therefore, responses may depend on the underlying cycle and the time of hormone infusion. Certainly modification of the pyloric and gastric rhythms of *C. borealis* by F1 or F2 depends on the original state of each of these rhythms (Weimann *et al.*, 1993). At F1 or F2 concentrations of 10^{-7} mol · l^{-1} and above, hemolymph flow through the anterolateral arteries of *C. magister* often did not regain pretreatment capacity until 3–6 h after hormone infusion (unpubl. obs.). The cardioarterial valves of the anterolateral vessels in *P. clarkii* show a persistent increase in resistance to F1 (J. L. Wilkens, pers. comm.). Thus it is possible that long-term contraction of the valves in *C. magister* also decreased blood flow through these arteries.

The paired hepatic arteries, which perfuse the hepatopancreas (Pearson, 1908; McLaughlin, 1983), were the only vessels that did not show an initial increase in flow. However, these arteries recovered pretreatment levels of flow before the other systems, and therefore most of the cardiac output was diverted to the digestive gland (Fig. 7a and b). The FaRPs are thought to be implicated in digestive processes in crustaceans (Mercier *et al.*, 1991; J. L. Wilkens, pers. comm.). They initiate pyloric and gastric rhythms in *C. borealis* (Weimann *et al.*, 1993) and act on the cardioarterial valves of the lobster to increase cardiac output to vessels supplying the gut; they also increase hindgut motility in *P. clarkii* (J. L. Wilkens, pers. comm.). This explanation does not, however, account for the long-term decrease in perfusion of the anterolateral arteries that supply the foregut of *C. magister* (Pearson, 1908; McLaughlin, 1983). A further anomaly is echoed in work on the isolated heart of the crayfish *P. clarkii*, in which the hepatic artery valves contract in response to F1 administration and thus decrease perfusion of the hepatopancreas (J. L. Wilkens, pers. comm.).

The overall effect, therefore, of F1 and F2 in *C. magister* is to induce a temporary increase in perfusion of the locomotory structures, mouthparts, CNS, brain, and telson *via* the sternal, anterolateral, anterior, and posterior arterial systems (Fig. 7a, b, c). These structures are thus perfused either periodically (Figs. 4 and 5) or at a lower rate, and the largest part of a reduced cardiac output is delivered to the hepatopancreas *via* the hepatic arteries (Fig. 7b, c). The functional significance of this is unclear; however, the overall pattern is not dissimilar to that characteristic of burrowing, when hemolymph is diverted to the locomotory structures and mouthparts. Once submerged in the mud, crabs become quiescent and perfuse the system only periodically (McGaw and McMahon, unpubl. obs.). While an animal is burrowed, channeling cardiac output to the hepatopancreas may aid digestion, which implicates FaRPs in a postprandial role in crustaceans.

In the present study, F1 was about 10 times more potent than F2 (Fig. 6), although the activity of these two peptides differs amongst the decapod crustaceans and appears to depend upon the species under investigation (Krajniak, 1991; Mercier and Russenes, 1992; Wilkens and McMahon, 1992; J. L. Wilkens, pers. comm.).

The function of the FaRPs is still not fully understood—in other invertebrate phyla their action on the cardiovascular system is variable, and excitatory, inhibitory, or biphasic responses vary with the concentration of the hormone or the species of organism (*e.g.*, Painter and Greenberg, 1982; Cuthbert and Evans, 1989; Price *et al.*, 1990; Duve *et al.*, 1993; Lesser and Greenberg, 1993). Future work will focus on the sites of action of these peptides to ascertain their role in cardioregulation, and measurement of circulating levels will determine whether they are released from the pericardial organs in amounts sufficient to effect the reported changes.

Acknowledgments

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Literature Cited

Airriess, C. N., and B. R. McMahon. 1992. Aminergic modulation of circulatory performance in the crab, *Cancer magister*. Pp. 123–131 in *Comparative Physiology*, Vol 11, R. B. Hill, K. Kuwasawa, B. R. McMahon, and T. Kuramoto, eds. Karger, Basel.

Airriess, C. N., and B. R. McMahon. 1994. Cardiovascular adaptations enhance tolerance of hypoxia exposure in the crab *Cancer magister*. *J. Exp. Biol.* **190:** 23–41.

Airriess, C. N., B. R. McMahon, I. J. McGaw, and G. B. Bourne. 1994. Application and *in situ* calibration of a pulsed-Doppler flowmeter for blood flow measurements in crustaceans. *J. Mar. Biol. Ass. U.K.* **74:** 455–458.

Cuthbert, B. A., and P. D. Evans. 1989. A comparison of the effects of FMRFamide-like peptides on locust heart and skeletal muscle. *J. Exp. Biol.* **144:** 395–415.

Duve, H., A. J. Elia, I. Orchard, A. H. Johnsen, and A. Thorpe. 1993. The effects of CalliFMRFamides and other FMRFamide-related neuropeptides on the activity of the heart of the blowfly *Calliphora vomitoria*. *J. Insect Physiol.* **39:** 31–40.

Greenberg, M. J., and D. A. Price. 1983. Invertebrate neuropeptides: native and naturalized. *Ann. Rev. Physiol.* **45:** 271–288.

Groome, J. R., and W. H. Watson III. 1989. Second messenger systems underlying amine and peptide actions on cardiac muscle in the horseshoe crab *Limulus polyphemus*. *J. Exp. Biol.* **145:** 419–438.

Kobierski, L. A., B. S. Beltz, B. A. Trimmer, and E. A. Kravitz. 1987. FMRFamide-like peptides of *Homarus americanus*: distribution, immunocytochemical mapping, and ultrastructural localization in the terminal varicosities. *J. Comp. Neurol.* **266:** 1–15.

Krajniak, K. G. 1991. The identification and structure-activity relations of a cardioactive FMRFamide-related peptide from the blue crab *Callinectes sapidus*. *Peptides* **12:** 1295–1302.

Kravitz, E. A., L. Kobierski, B. A. Trimmer, and M. F. Goy. 1987. Peptide F1: a myoactive lobster peptide related to FMRFamide. *Soc. Neurosci. Abstr.* **13:** 1257.

Lesser, W., and M. J. Greenberg. 1993. Cardiac regulation by endogenous small cardioactive peptides and FMRFamide-related peptides in the snail *Helix aspersa*. *J. Exp. Biol* **178:** 205–230.

Marder, E., R. L. Calabrese, M. P. Nusbaum, and B. Trimmer. 1987. Distribution and partial characterization of FMRFamide-like peptides in the stomatogastric nervous systems of the rock crab *Cancer borealis*, and the spiny lobster *Panulirus interruptus*. *J Comp. Neurol* **259:** 150–163.

McGaw, I. J., C. N. Airriess, and B. R. McMahon. 1994a. Peptidergic modulation of cardiovascular dynamics in the Dungeness crab *Cancer magister*. *J. Comp. Physiol. B.* **164:** 103–111.

McGaw, I. J., C. N. Airriess, and B. R. McMahon. 1994b. Patterns of hemolymph flow variation in decapod crustaceans. *Mar. Biol* **121:** 53–60.

McLaughlin, P. A. 1983. Internal anatomy. Pp. 1–41 in *The Biology of Crustacea*, Vol 5, L. H. Mantel, ed. Academic Press, New York.

McMahon, B. R., and C. L. Reiber. 1991. Peptidergic modulation of hemolymph flow in the lobster *Homarus americanus*. *FASEB J* **5(6):** 3390A.

Mercier, A. J., and R. T. Russenes. 1992. Modulation of crayfish hearts by FMRFamide-related peptides. *Biol. Bull.* **182:** 333–340.

Mercier, A. J., I. Orchard, and V. TeBrugge. 1991. FMRFamide-like immunoreactivity in the crayfish nervous system. *J. Exp. Biol* **156:** 519–538.

Morris, S., and B. R. McMahon. 1989. Neurohumor effects on crustacean haemocyanin oxygen affinity. *J. Exp. Zool.* **249:** 334–337.

Painter, S. D., and M. J. Greenberg. 1982. A survey of responses of bivalve hearts to the molluscan neuropeptide FMRFamide and to 5-hydroxytryptamine. *Biol. Bull.* **162:** 311–332.

Pasztor, V. M., and L. B. Golas. 1993. The modulatory effects of serotonin, neuropeptide F1 and proctolin on the receptor muscles of the lobster abdominal stretch receptor and their exoskeletal muscle homologues. *J. Exp. Biol* **174:** 363–374.

Pearson, J. 1908. Cancer. *Liverpool Mar. Biol. Comm. Mem.* **16:** 1–209.

Potvin, C., M. J. Lechowicz, and S. Tardif. 1990. The statistical analysis of ecophysiological responses curves obtained from experiments involving related measures. *Ecology* **71:** 1389–1400.

Price, D. A., and M. J. Greenberg. 1977. Purification and characterization of a cardioexcitatory neuropeptide from the central ganglia of a bivalve mollusc. *Prep. Biochem.* **7:** 261–281.

Price, D. A., and M. J. Greenberg. 1989. The hunting of the FaRPs: the distribution of FMRFamide-related peptides. *Biol. Bull.* **177:** 198–205.

Price, D. A., W. Lesser, T. D. Lee, K. E. Doble, and M. J. Greenberg. 1990. Seven FMRFamide-related and two SCP-related cardioactive peptides from Helix. *J. Exp. Biol.* **154:** 421–437.

Raffa, R. B. 1988. The action of FMRFamide (Phe-Met-Arg-Phe-NH_2) and related peptides on mammals. *Peptides* **9:** 915–922.

Trimmer, B. A., L. A. Kobierski, and E. A. Kravitz. 1987. Purification and characterization of FMRFamide-like immunoreactive substances from the lobster nervous system: isolation and sequence analysis of two closely related peptides. *J. Comp. Neurol.* **266:** 16–26.

Weimann, J. M., E. Marder, B. Evans, and R. L. Calabrese. 1993. The effects of SDRNFLFRamide and TNRNFLFRamide on the motor patterns of the stomatogastric ganglion of the crab *Cancer borealis*. *J. Exp. Biol* **181:** 1–26.

Wilkens, J. L., and B. R. McMahon. 1992. Intrinsic properties and extrinsic neurohormonal control of crab cardiac hemodynamics. *Experientia* **48:** 827–833.

Zar, J. H. 1984. *Biostatistical Analysis* Prentice-Hall, Englewood Cliffs, NJ.